Exposure of *Xenopus laevis* Tadpoles to Cadmium Reveals Concentration-dependent Bimodal Effects on Growth and Monotonic Effects on Development and Thyroid Gland Activity

Bibek Sharma* and Reynaldo Patiño†

*Department of Natural Resources Management; and †USGS Texas Cooperative Fish and Wildlife Research Unit, Texas Tech University, Lubbock, Texas 79409-2120

Received March 7, 2008; accepted June 10, 2008

*Xenopus laevis* were exposed to 0–855 μg cadmium (Cd)/l (measured concentrations) in FETAX medium from fertilization to 47 days postfertilization. Measurements included embryonic survival and, at 47 days, tadpole survival, snout-vent length, tail length, total length, hindlimb length, weight, Nieuwkoop-Faber (NF) stage of development, initiation of metamorphic climax (≥ NF 58), and thyroid follicle cell height. Embryonic and larval survival were unaffected by Cd. Relative to control tadpoles, reduced tail and total length were observed at 0.1–8 and at 855 μg Cd/l; and reduced snout-vent length, hindlimb length, and weight were observed at 0.1–1 and at 855 μg Cd/l. Mean stage of development and rate of initiation of climax were unaffected by Cd at 0–84 μg/l; however, none of the tadpoles exposed to 855 μg Cd/l progressed beyond mid-premetamorphosis (NF 51). Thyroid glands with fully formed follicles were observed in all tadpoles ≥ NF 49 examined. Follicle cell height was unaffected by Cd at 0–84 μg/l; in the latter, cell height was reduced even when compared with NF 49–51 tadpoles pooled from the 0 to 84 μg Cd/l groups. In conclusion, (1) Cd affected tadpole growth in a bimodal pattern with the first and second inhibitory modes at concentrations below and above 84 μg Cd/l, respectively; (2) exposure to high Cd concentrations (855 μg/l) reduced thyroid activity and arrested tadpole development at mid-premetamorphosis; and (3) unlike its effect on growth, Cd inhibited tadpole development and thyroid function in a seemingly monotonic pattern.

**Key Words:** cadmium; growth; metamorphosis; thyroid; amphibian.

Amphibian populations seem to be declining globally—most notably in protected areas (Halliday, 2005; Houlahan et al., 2000). Among the major factors which have been hypothesized for amphibian declines (Collins and Storfer, 2003), habitat degradation due to chemical contamination has been at least partly implicated (Bögí et al., 2003; Carr et al., 2003a, b; Clark et al., 1998; Goleman et al., 2002a, b; Hayes et al., 2003; Iwamuro et al., 2003; Levy et al., 2004; Qin et al., 2003). Cadmium (Cd) is a heavy metal found in natural surface waters of the United States typically at concentrations less than 0.1 μg/l (U.S. Environmental Protection Agency, 2001). This metal is also anthropogenically released into the environment by the combustion of fossil fuels, smelter operations, use of certain fertilizers in agriculture, and mining operations (U.S. Department of Health and Human Services, 1999). Cadmium values as high as 14–60 μg/l have been detected in urban or highway stormwater runoff (U.S. Department of Health and Human Services, 1999). Relatively high levels of Cd (up to 14 μg/l) were recently detected in surface waters of amphibian habitat in the Big Bend region of the Rio Grande Basin, Texas (R. Patiño et al., unpublished data), although the source of this Cd is uncertain. The U.S. Environmental Protection Agency (2006) has established a safety level (Criterion Continuous Concentration) of 0.25 μg Cd/l (at total hardness of 100 mg CaCO3/l) for the protection of freshwater aquatic life.

The toxicity of Cd has been extensively studied in freshwater teleosts (Spry and Wiener, 1991). In addition to modifying the activity of enzymes in several organs such as liver, kidney, and gut (Gill et al., 1991), Cd has been associated with inhibition of gametogenesis and developmental abnormalities in many teleost species (Gill and Pant, 1983; Ricard et al., 1998; Vetillard and Bailhache, 2005). Relatively little attention has been placed on the sublethal effects of heavy metals at low levels on growth and development of amphibians. Furthermore, the few studies of amphibians that are available have shown species-specific response patterns to Cd exposures. Herkovits et al. (1997) observed reduced body size and other somatic abnormalities during early tadpole stages of the African clawed frog, *Xenopus laevis*, when exposed to Cd concentrations ranging from 100 μg/l to very high levels (up to 10,000 μg/l). James et al. (2003) reported decreased survival and metamorphosis in American toad (*Bufo americanus*) during chronic exposure to Cd at a concentration of 540 μg/l, but observed increased growth at 5 and 54 μg/l and increased metamorphosis at 54 μg/l.
In an outdoor mesocosms study, James et al. (2005) found that percent survival and metamorphosis were significantly reduced at Cd concentrations of 60 μg/l and above for American toads and at 18 μg/l and above for southern leopard frogs (Rana sphenocephala); but they did not observe the stimulatory effect of Cd on development that was recorded in the earlier laboratory study with American toad (James et al., 2003). In a study with northern leopard frog (Rana pipiens), Gross et al. (2007) observed stimulation of tadpole growth and metamorphosis at Cd concentrations of 0.25–5 μg/l. Overall, the results of previous studies of Cd effects on amphibian development are insufficient to draw general conclusions or to define a consistent pattern.

There are several studies indicating that Cd can be toxic to the thyroid endocrine system of several vertebrates, including rodents and teleosts (Jadhao et al., 1994; Ricard et al., 1998; Wade et al., 2002; Yoshida et al., 1987). A decrease in whole-body triiodothyronine levels in tadpoles of X. laevis following a 14-day exposure to Cd also has been reported (Fort et al., 2000). Although the thyroid endocrine system is key to amphibian metamorphosis (Fort et al., 2007; Shi, 2000), knowledge of the effects and mechanisms of Cd on the amphibian thyroid gland is limited.

The cause of amphibian declines is likely to be a complex interaction of various anthropogenic factors. However, because of their wide geographic distribution, a better understanding of the sublethal effects of metal contaminants on amphibian growth and development would be useful to studies of ecological risk assessment. The objective of the present study is to determine the effects of Cd at low concentrations on growth, development and thyroid gland condition of African clawed frog tadpoles.

**MATERIAL AND METHODS**

**Animals.** Sexually mature male and female X. laevis (Xenopus Express, Homosassa, FL) were held at 12:12-h light/dark regimens and 20 ± 2°C and maintained in 40-l glass aquaria containing dechlorinated tap water (median pH: 7.6 and salinity: 0.8 g/l). Fifty percent of the water volume was replaced daily. They were fed frog brittle (Nasco, Ft Atkinson, WI) daily (three to four brittle nuggets per frog). Frogs were induced to breed following the method described by Goleman et al. (2002a). Briefly, five male-female pairs were each allowed to acclimate for 7 days in respective 40-l glass aquaria containing 18 l of Frog Embryo Teratogenesis Assay—Xenopus (FETAX) medium. Fifty percent of the FETAX medium was replaced daily. Spawning was induced by injecting both males and females via the dorsal lymph sac with human chorionic gonadotropin (hCG, Sigma Chemical, St. Louis, MO) at 1 g/l, and rinsed in distilled water. The animals were staged, weighted and measured for snout-to-vent length (SVL), tail length, total length, and hindlimb length (HLL). Three of the samples collected from each tank were randomly set aside for whole-body Cd analysis. These samples were wrapped in aluminum foil and stored at −80°C until analysis. The other 10 animals from each tank were set aside for histopathological analysis. These samples were placed in Bouin’s fixative (EMD Chemicals, Inc., Gibbstown, NJ) for 48 h followed by a 24-h rinse in tap water and stored in 70% ethanol until analysis.

Developmental stage (NF [Nieuwkoop-Faber] stage) was assigned according to Nieuwkoop and Faber (1994). Stages were classified into premetamorphosis (NF 46–54), prometamorphosis (NF 55–57), and metamorphic climax (NF 58–65) according to Shi (2000). Forelimb emergence begins at NF 58 and was used as index of the initiation of metamorphic climax.

**Water quality.** Water conditions of pH, dissolved oxygen (DO), salinity and specific conductivity were monitored twice weekly, once on Sunday (the day before water exchange) and the other time on Friday (a day after water exchange); unionized ammonia once weekly, on Friday; and temperature daily. A YSI 85 meter (Yellow Springs, OH) was used to monitor water temperature, DO, and conductance; an Oakton pH meter (Gresham, OR) to measure pH; and a Hach spectrophotometer model DR/1000 (Loveland, CO) to measure total ammonia according to manufacturer procedures. Unionized ammonia was calculated from total ammonia-nitrogen based upon temperature and pH (Emerson et al., 1975).

**Preparation of exposure solutions and analysis.** A master stock solution of 100 mg Cd/l was prepared once a day by dissolving 0.16 g of CdCl2 (CAS No: 10108-64-2, > 99% purity; Fisher Scientific, Waltham, MA) in 1 l of FETAX media. The stock solution was kept in Environmental Protection Agency-recommended, amber-colored glass vials and stored at 4°C. Serial dilutions of this stock solution were conducted to obtain the target nominal concentrations of Cd in treatment medium immediately before each water exchange.

Cadmium levels in exposure media were monitored through the entire duration of the study. A pilot trial indicated that Cd levels in the present test system are stable for at least 4 days (B. Sharma, unpublished observations). Thus, aliquots of water from control and Cd-treated aquaria were collected into amber-colored bottles only after each water exchange with the expectation that the Cd levels would not change significantly between water exchanges. The aliquots were fixed in approximately 0.1% trace metal-grade nitric acid, stored at 4°C and analyzed for Cd concentration within three days of collection. Samples were fixed in approximately 0.1% trace metal-grade nitric acid, stored at 4°C until analysis. The other 10 animals from each tank were set aside for histopathological analysis. These samples were placed in Bouin’s fixative (EMD Chemicals, Inc., Gibbstown, NJ) for 48 h followed by a 24-h rinse in tap water and stored in 70% ethanol until analysis.

**Experimental design.** Naturally fertilized eggs were obtained from the three of the breeding pairs. All the embryos obtained from those breeding pairs we pooled and then embryos for the study were selected randomly from the pool. Within 24 h of fertilization, 60 embryos were transferred to individual 20-l glass aquaria containing 8 l of FETAX medium (water depth in the tank was approximately 17 cm) with 0, 0.1, 1, 10, 100, and 1000 μg Cd/l (nominal concentrations). Each treatment was conducted in quadruplicate and all tanks were maintained at about 22°C on a 12:12-h light/dark regime with continuous, gentle aeration throughout the exposure. Embryos were allowed to hatch for a period of 96 h. During this time interval dead embryos, if any, were removed twice daily. The data collected on dead embryos was used to compute embryonic survival. After hatching, daily records were kept for larval mortality to compute the posthatching (larval) survival. Starting at day 5 posthatch, larvae were fed ad libitum powdered frog brittle made into a paste with FETAX medium. Tanks were monitored daily for feed depletion and additional feed provided as needed. To prevent the possibility of incorporating feeding bias, feed from all tanks were removed before providing any new installment of feed ration. Fifty percent static-renewal of exposure media was conducted twice a week (Monday and Thursday) on all aquaria. Daily records were maintained for hatching success, embryo, and larval mortality, and animals showing initiation of metamorphic climax (forelimb emergence).

On day 47 of treatment, when it became evident that a number of tadpoles in all control tanks had either initiated climax or were approaching this stage, thirteen tadpoles were collected randomly from each treatment tank (total of 52 per treatment). All the sampled tadpoles were euthanized by immersing in 3-aminobenzoic acid ethyl ester (MS-222, Sigma-Aldrich Chemical, St Louis, MO) 1 g/l, and rinsed in distilled water. The animals were staged, weighed and measured for snout-to-vent length (SVL), tail length, total length, and hindlimb length (HLL). Three of the samples collected from each tank were randomly set aside for whole-body Cd analysis. These samples were wrapped in aluminum foil and stored at −80°C until analysis. The other 10 animals from each tank were set aside for histopathological analysis. These samples were placed in Bouin’s fixative (EMD Chemicals, Inc., Gibbstown, NJ) for 48 h followed by a 24-h rinse in tap water and stored in 70% ethanol until analysis.

Developmental stage (NF [Nieuwkoop-Faber] stage) was assigned according to Nieuwkoop and Faber (1994). Stages were classified into premetamorphosis (NF 46–54), prometamorphosis (NF 55–57), and metamorphic climax (NF 58–65) according to Shi (2000). Forelimb emergence begins at NF 58 and was used as index of the initiation of metamorphic climax.

**Laboratory study with American toad (James et al., 2003). In a study with northern leopard frog (Rana pipiens), Gross et al. (2007) observed stimulation of tadpole growth and metamorphosis at Cd concentrations of 0.25–5 μg/l. Overall, the results of previous studies of Cd effects on amphibian development are insufficient to draw general conclusions or to define a consistent pattern.**
were analyzed using a GF95 Graphite Furnace Atomic Absorption (GF-AA) Series Spectrometer (Thermo Electron Corporation, Waltham, MA). Standard curves were established by measuring different dilutions of a Cd standard (SPEXCertiprep; Metuchen, NJ), the lowest dilution being 0.05 µg Cd/l. The accuracy and integrity of the sample analysis was monitored by regularly running check standards and deionized water blanks. The limit of detection (LOD) was calculated according to the formula: LOD = (mean value of the blank) + 2 (standard deviations of the blank). The limit of quantitation (LOQ) was calculated according to the formula: LOQ = LOD × [1/(relative standard deviation of the standard curve)]. The LOD and LOQ for this analysis were 0.020 µg and 0.060 µg Cd/l, respectively.

Whole-body cadmium analysis. All samples from each treatment were pooled to yield a composite sample (n = 3 tadpoles per replicate; total per treatment pool = 12 animals). Pooled samples were pulverized in liquid nitrogen, mixed and allowed to air-dry overnight at room temperature to constant weight. The dried samples were then acid-digested at 95 ± 5°C with trace metal-grade nitric acid until complete mineralization. After mineralization the samples were allowed to cool and then were filtered through Whatman no. 41 filter paper, diluted to 25 ml with deionized water, and analyzed by GF-AA. A TORT-2 lobster hepatopancreas reference material (National Research Council of Canada, Institute of National Measurement Standards, Ontario, Canada) with known metal concentrations was digested and processed simultaneously with the samples to assess extraction efficiency and to correct the measured values for losses. Development of standard curves and other procedures (e.g., use of check standards and deionized water blanks) were as described for water analysis. The LOQ for extracted samples was calculated according to the formula, LOQ = (lowest calibration concentration) x (volume of the extract/mass or volume of the sample). The LOQ for whole-body Cd measurements was 3.125 µg Cd/kg.

Thyroid histopathology. A previous study reported the presence of colloid in thyroid follicles of X. laevis at NF 49 (Hu et al., 2006), suggesting that the thyroid gland becomes functional at or near this stage of development. Thus, thyroid analyses for this study were conducted only on tadpoles at NF 49 and above. Tissue processing procedures were according to Carr et al. (2003a). The lower jaw was isolated and used to prepare blocks of paraffin. Sections were cut at 6-μm thickness and stained with hematoxylin and eosin. Histological sections cutting through the middle of thyroid glands were selected for analysis. Images of the thyroid follicles were taken with an Olympus digital camera (DP70; Tokyo, Japan) attached to a compound microscope. Thyroid follicle cell height measurements were conducted using ImagePro Express Software (Media Cybernetics, Silver Spring, MD) following the procedures of Mukhi et al. (2005). Briefly, epithelial cell height was measured at four predetermined positions (12:00, 3:00, 6:00, and 9:00 A.M.) in each of three to seven follicles per frog. Mean cell height was calculated for each follicle, and the mean of all measured follicles was determined for each animal.

Data analysis. The unit of replication was the individual tadpole for stage of development, growth and thyroid endpoints; and the tank for percent survival and percent onset of metamorphic climax. Homogeneity of variances (for ANOVA) was assessed using Bartlett’s test, and Gaussian distributions (for Student’s t-test) were examined using Shapiro-Wilk normality test. Data such as rates of survival and initiation of metamorphic climax were transformed to arcsine of square root values before analysis. One-way nested ANOVA (unit of replication, individual) or one-way ANOVA (unit of replication, tank) followed by the Tukey-Kramer multiple comparison test were used when the assumptions of parametric statistics were met. If these assumptions were not met, data were subjected to a Randomization test with 1000 iterations followed by Student’s t-tests with Welch’s correction and Bonferroni’s adjustments. An overall n value of 0.05 was used to assess significant differences. Data are reported as mean ± SEM. Most statistical analyses were conducted with STATISTICA data mining software, version 8 (StatSoft, Inc., Tulsa, OK). Randomization tests were conducted using Resampling Stats software, Version 3.2 (Resampling Stats, Arlington, VA).

RESULTS

Water Quality

Mean water temperature, pH, conductivity and DO were 21.1°C (range, 20.3–21.8°C), 7.9 (range, 7.4–8.2), 1565 µS/cm (range, 1522–1785 µS/cm), and 6.7 mg/l (range, 4.9–7.1 mg/l). Unionized ammonia ranged from 0 to 0.035 mg/l over the 47-day exposure.

Water and Whole-Body Cadmium Concentrations

Mean (± SEM) Cd concentrations in tank water samples collected through the entire exposure period were 0 ± 0.09 ± 0.03, 0.79 ± 0.12, 8.3 ± 0.86, 83.5 ± 6.9, and 854.6 ± 40.5 µg Cd/l, respectively, for the 0, 0.1, 1, 10 and 1000 µg Cd/l nominal concentrations. Chi-square analysis (df = 5, p < 0.01) indicated significant differences between the nominal and measured Cd concentrations; thus, mean values rounded to nearest decimal places are reported as the exposure concentrations: 0, 0.1, 1, 8, 84, and 855 µg Cd/l. Whole-body contents of Cd were 0.82, 2.5, 6.6, 8.4, 14, and 100 µg/g (dry weight) for the 0, 0.1, 1, 8, 84, and 855 µg/l exposure treatments, respectively. Although the present data are insufficient for reliable regression analysis, the relationship between water and whole-body Cd concentration was clearly not linear (Fig. 1).

Survival, Growth, and Development

Embryonic and larval survival rates were not significantly affected at any of the Cd concentrations tested (one-way ANOVA, p > 0.05). The mean (± SEM) embryonic survival was 90 ± 1.1, 90 ± 2.3, 94 ± 0.6, 92.5 ± 3.3, 85.5 ± 2.3, and 87 ± 3.1% for the 0, 0.1, 1, 8, 84, and 855 µg Cd/l exposure concentrations, respectively. The mean (± SEM) posthatch survival was 83.6 ± 1.7, 78.6 ± 3.1, 85.4 ± 2.9, 78.4 ± 2.2, 87.2 ± 2.2, and 73.7 ± 5.6% for the 0, 0.1, 1, 8, 84, and 855 µg Cd/l treatment concentrations, respectively.

FIG. 1. Semilogarithmic plot of Cd water concentration (µg/l) and whole-body content (µg Cd/g dry weight) of Xenopus tadpoles at the end of a 47-day exposure starting at fertilization.
All growth parameters measured showed a concentration-dependent bimodal response to Cd. Significant reductions in tail length and total length relative to untreated controls were observed at 0.1, 1, 8, and 855 but not at 84 μg Cd/l (Randomization test and Student’s t-tests with Welch’s and Bonferroni’s corrections, p < 0.05) (Fig. 2). Similarly, reduced SVL, HLL, and weight were observed at 0.1, 1, and 855 but not at 8 or 84 μg Cd/l (Randomization test and Student’s t-tests with Welch’s and Bonferroni’s corrections, p < 0.05) (Fig. 2).

Mean stage of development and percent onset of metamorphic climax (Fig. 3) were not significantly affected at Cd concentrations of 0.1–84 μg/l relative to untreated controls (Randomization test and Student’s t-tests with Welch’s and Bonferroni’s corrections, p > 0.05). The range of stages were NF 49–63, 48–65, 49–62, 49–64, 49–64, and 48–51 in control, 0.1, 1, 8, 84, and 855 μg Cd/l, respectively.

**Thyroid Gland Condition**

Thyroid follicles in tadpoles from all treatments were lined with a single layer of epithelial cells and pinocytotic vesicles of colloid were observed at the periphery of the lumen (Fig. 4). However, a clear difference in the size of the thyroid gland and follicles was evident in most tadpoles of the 0–84 versus the 855 μg Cd/l treatment groups (see next paragraph). When all tadpoles examined were included in the analysis, a significant difference (reduction) in follicle cell height was observed only in tadpoles exposed to 855 μg Cd/l (one-way nested ANOVA and Tukey-Kramer multiple comparison test, p < 0.001) (Fig. 5). No tank effects were noted in this analysis (p > 0.05).

Thyroid follicle cell height is associated with stage of development during metamorphosis (e.g., Hu et al., 2006). Thus, to account for the confounding effect of the difference in mean stage of development between tadpoles exposed to 855 μg Cd/l and the other Cd treatments, we also compared thyroid epithelial cell height in tadpoles (NF 49–51) from the 855 μg Cd/l treatment group (n = 33) against the values obtained from pooled NF 49 to 51 tadpoles in all other groups (0–84 μg Cd/l; n = 16). A two-sided Student’s t-test (with Welch’s correction) showed a significant difference in mean epithelial cell height between these two groups of tadpoles (p < 0.05) (Fig. 5).

**DISCUSSION**

The most common endpoint used by earlier studies of the toxicity of Cd in amphibians is survival (Linder and Grillitsch, 2000). In the present study, embryonic and tadpole survival in *X. laevis* were not affected by exposure to Cd at any of the measured concentrations used (0–855 μg/l) up to 47 days of exposure. Embryo survival was 85.5% or greater in all treatments (90% in control animals), and tadpole survival was 74% or greater in all treatments (83.6% in control animals). An earlier study with *X. laevis* reported an embryonic LC50 for Cd-chloride of 850 μg/l (Linder et al., 1991); a finding that seems inconsistent with the results of the present study. However, one reason for this difference in embryonic mortalities...
may be the hardness of the test media used: 40–100 mg/l in the study of Linder et al. (1991; and G. Linder, personal communication) and 170 mg/l in the present study. [The toxicity of cadmium to aquatic organisms correlates negatively with water hardness (U.S. Environmental Protection Agency, 2001).] Using test media with the same hardness level as in the present study (170 mg/l), Canton and Slooff (1982) reported LC50 and NOEL values for mortality of 1500 and 30 μg/l, respectively, for tadpoles of X. laevis following an exposure period of 100 days. The larval LC50 for Cd could not be calculated in the present study due to the lack of treatment-associated mortality, but the length of the exposure period was one-half of that in the study of Canton and Slooff (1982). The toxicity of Cd at relatively low concentrations has been recently reported for the early life stages of other amphibians. Gross et al. (2007) observed no effect of Cd exposure on the embryonic survival of northern leopard frog at concentrations up to 20 μg/l (nominal; total hardness, 170 mg/l), but they found that larval survival was greatly reduced after 56–63 days of exposure to 20 μg Cd/l (nominal). James et al. (2003) found a significant decline in larval survival of American toad exposed (posthatch) to Cd at 540 μg/l (measured concentration; total hardness, 50 mg/l) but not at 5 or 54 μg/l. In another study using outdoor mesocosms, James et al. (2005) reported that larval survival of American toad tadpoles was reduced at 60 μg/l and higher Cd concentrations (nominal; total hardness, 60 mg/l), and that of southern leopard frog tadpoles at 18 μg/l and higher concentrations (nominal; total hardness, 60 mg/l).

Overall, the results of the present and previous studies are consistent with the view that the level of Cd toxicity in amphibians is species dependent. Compared with other amphibians examined to date, embryos and tadpoles of X. laevis seem to be relatively tolerant to Cd exposure in terms of survival. In fact, the current chronic level criterion for Cd in freshwater is 0.35 μg/l (at FETAX hardness of 170 mg/l; U.S. Environmental Protection Agency, 2006), which is well below lethal levels for X. laevis embryos and larvae at the Cd concentrations and other exposure conditions of the present study. In contrast, sublethal growth endpoints seemed to be very sensitive to Cd exposure in Xenopus tadpoles.

All tadpole growth endpoints examined in the present study showed a bimodal response to increasing Cd exposure concentrations, with the first and second (inhibitory) modes at concentrations below and above 84 μg/l, respectively. Relative to control values, reduced tail length and total length were observed at 0.1, 1, 8, and 855 μg Cd/l; and reduced SVL,

FIG. 3. Mean stage of development (A), and percent forelimb emergence (onset of metamorphic climax) (B) in tadpoles of Xenopus laevis exposed to untreated FETAX medium or medium containing Cd at various measured concentrations from fertilization to day 47. Bars represent the mean values (±SEM). Bars with common letters are not significantly different (Randomization test and Student’s t-tests with Welch’s and Bonferroni’s corrections, p < 0.05).

FIG. 4. Photomicrographs of thyroid glands from Xenopus laevis at 47 days after fertilization. Thyroid follicles from control tadpoles (A: NF 58 in the picture) and those exposed to 0.1–84 μg Cd/l showed relatively tall columnar epithelium. Follicles from tadpoles exposed to 855 μg Cd/l (B: NF 50 in the picture) were relatively small and had relatively thinner epithelium. Asterisks show the lumens of several follicles within each thyroid gland. Bars = 50 μm.
HLL, and weight were observed at 0.1, 1, and 855 μg Cd/l. However, none of the growth parameters measured differed from control values at a Cd concentration of 84 μg/l (some of the endpoints were also not significantly different from control values at 8 μg Cd/l). These findings with X. laevis differ from those reported for northern leopard frog (Gross et al., 2007) and American toad (James et al., 2003), where tadpole growth seemed to be accelerated, not inhibited, relative to control values at low Cd concentrations: 5–54 μg/l (measured) and 0.25–5 μg Cd/l (nominal), respectively. However, there is one notable observation that is shared between the present study with X. laevis and the previous studies with R. pipiens (Gross et al., 2007) and B. americanus (James et al., 2003): all three laboratory studies showed increased tadpole growth within a defined range of low Cd concentrations—in the present study, from 0.1 to 84 μg/l. What is new and different in the present study with Xenopus tadpoles is the observation that, relative to unexposed controls, growth was inhibited at low Cd concentrations (below 84 μg/l). Thus, the difference between the present and the previous studies is in the growth response of unexposed (control) animals. Such bimodal growth response to varying concentrations of Cd is novel for amphibians and, to our knowledge, other vertebrates. (This observation has been repeated in a follow up study with X. laevis [B. Sharma and R. Patiño, unpublished data].) However, growth enhancing (James et al., 2003) and suppressing (James et al., 2005) effects of Cd relative to control animals have been previously reported for tadpoles of American toad. Although these studies with American toad were conducted with separate toad populations reared under different conditions (in the laboratory [James et al., 2003] or in outdoor mesocosms [James et al., 2005]), their combined results are consistent with the conclusion of the present study that Cd at low concentrations may inhibit or enhance tadpole growth relative to unexposed controls by as yet unknown mechanisms. It should also be noted that inhibitory effects of Cd on Xenopus tadpole growth were observed at 0.1 μg/l in the present study, a level that is below the current federal safety criterion of 0.35 μg Cd/l (at hardness of 170 mg/l) for the protection of freshwater aquatic life.

Stage of development and rate of metamorphic climax initiation seemed to show a slight trend for a biphasic response to Cd exposure, but these endpoints were significantly different (lower) only at 855 μg Cd/l. None of the animals exposed to 855 μg Cd/l were able to advance beyond NF 51 (hindlimb emergence) by the end of the 47-day exposure. Thyroid follicle cell height was also reduced in tadpoles at exposure concentrations of 855 μg Cd/l, even when the comparison was made against the subpopulation of NF 49–51 animals pooled from the 0 to 84 μg Cd/l groups. These observations indicate that the thyroid gland not only failed to activate at 855 μg Cd/l, but also that its activity was suppressed. Could thyroid dysfunction explain why tadpoles exposed to Cd at high concentration (855 μg Cd/l) failed to develop beyond mid-premetamorphosis? Although some investigators have suggested that thyroid hormone is not necessary for tadpole development during premetamorphosis (Fort et al., 2007; Shi, 2000)—thryocytes of premetamorphic Xenopus tadpoles seem to be able to synthesize thyroid hormone as early as NF 46 (Marsh-Armstrong et al., 2004), and fully formed follicles with colloid deposits are observed at NF 49 (Hu et al., 2006; present study). Moreover, certain developmental events of premetamorphosis, such as the generation of adequate numbers of spinal motor neurons, are under the control of thyroid hormone (Marsh-Armstrong et al., 2004). Thus, thyroid hormone seems to be not only produced but also necessary for normal development during premetamorphosis. Because Cd is not goitrogenic in X. laevis—it actually caused a mild reduction in follicle cell height (present study), Cd-dependent inhibition of thyroid function in Xenopus tadpoles (NF 60, Fort et al., 2000; NF 49–51, present study) may be caused by effects of Cd upstream of the thyroid gland, perhaps the brain or pituitary. This conclusion is supported by the observation that in the

FIG. 5. Thyroid follicle cell height in tadpoles of Xenopus laevis exposed to untreated FETAX medium or medium containing Cd at various measured concentrations from fertilization to day 47 (A); and thyroid follicle cell height in NF 49–51 tadpoles of X. laevis exposed to 0–84 μg Cd/l (pooled) or 855 μg Cd/l from fertilization to day 47 (B). Bars represent the mean values (±SEM). Bars associated with common letters are not significantly different (one-way ANOVA and Tukey-Kramer multiple comparison test; p < 0.05); asterisk, significantly different (unpaired Student’s t-test with Welch’s correction; two-tailed p < 0.05).
catfish Clarias batrachus, Cd exposure reduced the secretory activity of pituitary thyrotropes as well as the height of thyroid follicle cells (Jadhao et al., 1994). TSH transcripts have been detected in Xenopus pituitaries at NF 52 (Manzon and Denver, 2004); but synthesis and activity of TSH, and negative feedback regulation of the thyroid system may also occur at even earlier stages of premetamorphic development (reviewed by Manzon and Denver, 2004). Taken together, the preceding observations suggest that reduced thyroid gland activity caused by Cd at high exposure concentrations (855 μg/l) may be at least partly responsible for developmental arrest at premetamorphosis. Inhibition of metamorphic climax by Cd has been previously reported in X. laevis (Fort et al., 2000).

In addition to X. laevis (Fort et al., 2000; present study), Cd-dependent inhibition of metamorphosis has been reported in the newt, Pleurodeles waltl at Cd concentrations of 400 μg/l and higher (Flament et al., 2003); and in American toad at measured concentrations of 540 μg/l (James et al., 2003). However, Cd at low concentrations has been also reported to stimulate metamorphosis in American toad (at 54 μg/l; James et al., 2003) and northern leopard frog (at 0.25–20.0 μg/l; Gross et al., 2007). The regulation of tadpole growth and development is achieved via a complex interaction of various hormones (Fort et al., 2007), and the mechanisms by which Cd at low concentrations may stimulate metamorphosis in American toad (James et al., 2003) and northern leopard frog (Gross et al., 2007) are uncertain.

The Cd content of control tadpoles in the present study (0.82 μg/g dry weight) is similar to the Cd content reported in control American toad (1.5 μg/g dry weight) and southern leopard frogs (0.5 μg/g dry weight) metamorphs (James et al., 2005), and in control northern leopard frog (0.45 μg/g dry weight) tadpoles (Gross et al., 2007). As reported for northern leopard frog tadpoles (Gross et al., 2007), the association between measured water Cd concentrations and whole-body Cd content in Xenopus tadpoles was not linear. The results of the present study suggest that Xenopus tadpoles can tolerate whole-body Cd contents as high as 100 μg/g (dry weight)—reached after a 47-day exposure to a waterborne Cd concentration of 855 μg/l. However, these body Cd levels were associated with markedly reduced growth and with developmental arrest, and it is likely that longer exposure periods would have led to mortality.

In summary and conclusion, the present study documented a novel bimodal pattern of concentration-dependent effects of Cd on the larval growth of X. laevis; namely, inhibition of tadpole growth at low exposure concentrations (0.1–1–8 μg/l) was followed by restoration to control values ("stimulation") at 84 μg/l, and again by inhibition at 855 μg/l. Cadmium also affected development, but this effect appeared to follow a monotonic pattern of inhibition (observed only at the highest Cd concentration) and may have been partly caused by Cd-dependent thyroid dysfunction. Although Xenopus embryos and tadpoles are relatively tolerant to Cd in terms of surviving the exposures, the growth of Xenopus tadpoles seems to be relatively sensitive to low Cd exposure concentrations (0.1–8 μg/l).

Future studies of the developmental effects of Cd in amphibians should address intra- and interspecific variability in growth response patterns as well other ecologically relevant endpoints such as size at metamorphosis.

**FUNDING**

Texas Cooperative Fish and Wildlife Research Unit; and the Texas Tech University Association of Biologists.

**ACKNOWLEDGMENTS**

We acknowledge Drs George Cobb and Mike Hooper for their assistance during Cd analysis and Dylan Kuhe for his help with animal husbandry. Drs. Sandeep Mukhi and Greg Linder provided useful criticism on a draft version of this manuscript. Texas Cooperative Fish and Wildlife Research Unit cooperators include Texas Tech University, Texas Parks and Wildlife Department, U.S. Geological Survey, the Wildlife Management Institute, and U.S. Fish and Wildlife Service.

**REFERENCES**


